Progression of pectinate ligament dysplasia over time in two populations of Flat-Coated Retrievers

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Abstract: Objective Two of the authors (DG, BS) independently observed that a number of Flat-Coated Retrievers (FCRs) previously unaffected by pectinate ligament dysplasia (PLD) appeared to develop the condition later in life. This study was instigated to investigate progression of PLD within individual dogs over time. Animals studied Flat-Coated Retrievers that had previously undergone gonioscopy under the UK/ECVO hereditary eye schemes were included in the study. Procedure A second gonioscopic examination was performed 1.92–12.58 years later (mean 6, median 5.75 years) and the results compared. 39 FCR (17 males, 22 females) in the UK and 57 FCR (27 males, 30 females) in Switzerland were included. Slit-lamp biomicroscopy, indirect ophthalmoscopy, and gonioscopy were performed in all dogs. Gonioscopy allowed classification as either unaffected or affected; percentage of the iridocorneal drainage angle (ICA) affected by PLD was determined, before calculating progression observed as mild, moderate, or severe. Results 39 of 96 (40.6%) dogs demonstrated progression of PLD ($P < 0.0001$). Of these, 13 of 96 (13.5%) were classified as mild progression (from either unaffected to 10–20% or 10–20% to 20–90% ICA affected). Progression was more extensive in 26 of 96 (27.1%) dogs ($P < 0.0001$), of which 12 of 96 (12.5%) went from unaffected to severe PLD of >90% ICA affected, consistent with a high risk of glaucoma. Conclusions To the authors' knowledge, this is the first report describing progression of PLD in individual dogs over time, in a breed affected by primary, angle closure glaucoma. Key Words: Flat-Coated Retriever, goniodysgenesis, gonioscopy, iridocorneal angle, pectinate ligament dysplasia, primary glaucoma.

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EVOLUTION OF PECTINATE LIGAMENT DYSPLASIA OVER TIME IN TWO POPULATIONS OF FLATCOATED RETRIEVERS

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ABSTRACT

Objective: Two of the authors (DG, BS) independently observed that a number of Flatcoated Retrievers (FCRs) previously unaffected by pectinate ligament dysplasia (PLD) appeared to develop the condition later in life. This study was instigated to investigate progression of PLD within individual dogs over time.
Animals studied: FCRs that had previously undergone gonioscopy under the UK/ ECVO hereditary eye schemes were included in the study.
Procedure: A second gonioscopic examination was performed 1.92-12.58 years later (mean 6, median 5.75 years) and the results compared. 39 FCR (17 males, 22 females) in the UK and 57 FCR (27 males, 30 females) in Switzerland were included. Slit-lamp biomicroscopy, indirect ophthalmoscopy and gonioscopy were performed in all dogs. Gonioscopy allowed classification as either unaffected or affected; percentage of the iridocorneal drainage angle (ICA) affected by PLD was determined, before calculating progression observed as mild, moderate or severe. A paired t-test was used to determine if change was significant.
Results: 39/ 96 (40.6%) dogs demonstrated evolution of PLD ($p<0.0001$). Of these, 13/96 (13.5%) were classified as mild progression (from either unaffected to 10-20% or 10-20% to 20-90% ICA affected). Progression was more extensive in 26/96 (27.1%) dogs, ($P<0.0001$), of which 12/96 (12.5%) went from unaffected to severe PLD of >90% ICA affected, consistent with a high risk of glaucoma.
Conclusions: To the authors' knowledge, this is the first report describing evolution of PLD in individual dogs over time, in a breed affected by primary, angle closure glaucoma.
Keywords: Flatcoated retriever, pectinate ligament dysplasia, iridocorneal angle, gonioscopy, goniodysgenesis, primary glaucoma

INTRODUCTION

Glaucoma describes a final common pathway of a group of diseases which cause decreased retinal ganglion cell (RGC) sensitivity and function, RGC death, optic nerve axonal loss and concurrent optic nerve head cup enlargement with incremental reduction in visual fields and blindness[1]. The primary risk factor currently identified for these neurodegenerative diseases in the dog is an elevated intraocular pressure (IOP). Intra-ocular pressure is determined and maintained by the rate of aqueous humour formation, which equals rate of aqueous outflow[2].

Drainage of aqueous humour occurs at the iridocorneal angle (ICA). This anterior opening of the ciliary cleft is the outlet between the iris base and limbal cornea and is spanned by the comb-like pectinate ligament. Passage of aqueous humour through the pectinate ligament allows entry to uveal then corneoscleral trabecular meshworks for collection by the angular aqueous plexus before passing to the circulation via the intrascleral plexus and vortex venous drainage system[1]. Drainage via this conventional route accounts for 85% of aqueous outflow in the dog and the venous resistance created by this contributes to approximately 50-75% of the resistance that determines IOP (ii). The remaining 15% of aqueous outflow, draining via uveoscleral (or unconventional) outflow, is independent of IOP[2].

Primary glaucomas represent progressive diseases of the aqueous humour outflow pathways and develop in the absence of antecedent intra-ocular disease, whereas secondary glaucoma occurs when concurrent ocular disease obstructs aqueous outflow pathways[1]. Primary glaucoma in the dog has the potential for bilateral development and is considered hereditary in a number of breeds, including the Flatcoated Retriever[1]. Primary glaucomas may be subdivided according to the presence of an open or narrow ICA, either at gonioscopic examination or via imaging modalities such as ~~high resolution~~high-resolution ultrasonography or ultrasound biomicroscopy[1]. Pectinate ligament dysplasia (PLD) describes the consolidation of adjacent pectinate ligaments into broad sheets and is often reported in association with many primary narrow and closed angle glaucomas. Read *et al* (1989) demonstrated the presence of PLD to be significantly correlated to the risk of developing primary glaucoma in the Flatcoated Retriever breed and Wood *et al* demonstrated ~~an~~a hereditary basis for the presence of PLD in that same body of research[3, 4].

Gonioscopy refers to the clinical examination of the ICA and opening of the ciliary cleft[1]. Gonioscopic observations include subjective assessments of the width of ICA and the, length and diameter of pectinate ligaments along with description of any abnormalities. If PLD is identified then it may be semi-quantified in terms of quadrant or degree of ICA affected[1]. Gonioscopy has been recognised as essential to the evaluation of ICA abnormalities in the dog since studies published by both Martin in 1969 and Bedford in the 1970's[5-8]. Bedford examined a large number of dogs in the UK, including breeds considered at increased risk of primary glaucoma. He documented variations in the

pectinate ligament structure and angle width in the English Cocker Spaniel and Basset Hound at an age of 4 to 5 months and concluded these changes were likely congenital and not related to disease or ageing process[6]. Martin then went on to perform an early SEM study of the ICA morphology in puppies aged between 6 weeks pre-natal and 4 weeks post-natal and noted ongoing development of the pectinate ligament morphology during those early post-natal weeks[9]. This established a process of rarefaction of an initial sheet of ICA tissue, with progressive opening of intra-ligamentary spaces. Samuelson and Gelatt further clarified the histological detail of this process in a 1989 study of the ontogeny of the ICA in the normal Beagle[10]. Much rarefaction of the initial fibrillar sheet was complete by 2-4 weeks postnatal, leaving strands of intertwining collagen, progressively encased by attenuate trabecular cells, confluent with the anterior surface of the iris. Infrequent sheets, partially rarefied with holes, were however noted as late as 8 weeks postnatal and it was from 8 weeks of age onwards that morphological development of the pectinate ligament (and deeper angle structures) was considered complete.

In breeds for which ~~an~~a hereditary basis has been established for PLD, gonioscopy forms a component of national hereditary eye disease screening programmes and allows the identification of PLD-affected individuals prior to breeding, as well as indicating risk for developing glaucoma in individual dogs. National hereditary eye disease screening programmes in Europe include the British Veterinary Association/ Kennel Club/ International Sheep Dog Society (BVA/KC/ISDS) scheme and the European College of Veterinary Ophthalmologists Hereditary Eye Disease (ECVO HED) scheme. Subsequent to the developmental studies by Samuelson and Gelatt and in order to facilitate ease of examination with regard to the size of the eye, the UK (BVA/ KCS/ISDS) and ECVO hereditary eye panel certification has considered the gonioscopic examination to be a 'once in a lifetime' test, performed from 6 months of age onwards[11, 12].

This paper arose out of an observation that a number of individual adult Flatcoated Retriever dogs appeared to demonstrate progression of PLD over a period of time. This was noted independently by authors DJG and BS, in the UK and Switzerland respectively. To investigate this further, a joint study was undertaken to determine the incidence of PLD progression in representatives of the UK and Swiss populations of the Flatcoated Retriever breed over time. Flatcoated Retrievers that had previously undergone gonioscopy as part of the BVA/KC/ISDS or ECVO HED schemes were re-examined at a later age and gonioscopy was repeated. The results of initial and later gonioscopic findings were then compared to determine whether there had been progression of PLD over time.

MATERIALS AND METHOD

Two populations of Flatcoated Retrievers were examined, one cohort in the UK (FCR-UK) and the other in Switzerland (FCR-Swiss). The primary inclusion criteria was availability of gonioscopy data from a previous hereditary eye disease screening examination for each individual dog, under either the BVA/KC/ISDS eye panel in the UK or the ECVO HED scheme in Switzerland. Examinations on each of these dogs were then organised after enlisting the help of the Flatcoated Retriever breed club in each respective

country. In the UK, dogs attended a single event at which these second eye examinations took place, whilst in Switzerland dogs were invited to attend during regular clinic times and the second examinations took place over a longer time period. Data recorded included each individual dog's date of birth, sex, coat colour, kennel club registration number and permanent identification number (microchip or tattoo).

For FCR-UK dogs, initial gonioscopic examinations had been performed by a number of different BVA/KC/ISDS eye panellists, following the protocol of the BVA/KC/ISDS eye scheme (xi). The second gonioscopic examinations were performed by three experienced BVA/KC/ISDS eye panellists. Gonioscopy results from the first examination were masked from the examiners until after performing and recording second examination results. For FCR-Swiss dogs, both initial and second gonioscopic examinations were performed by one author (BS). Data from the first examination was again masked from the examiner until after the second examination.

Prior to gonioscopy, ophthalmic examination protocol included slit-lamp biomicroscopy, indirect ophthalmoscopy and direct ophthalmoscopy. Tonometry was not routinely performed unless indicated. The cornea was then anaesthetised with topical proxymetacaine (proparacaine) 0.5% (Bausch & Lamb, Chauvin Pharmaceuticals Ltd., France) prior to bilateral gonioscopic examination, performed as described by Read (iii). A Koeppe goniolens was used in all dogs, selecting sizes 17mm or 19mm in the UK dogs or 17mm, 18mm or 19mm in the Swiss dogs. Goniolens size was based on subjective assessment of corneal width and for the majority of these mature dogs a size 19mm was deemed appropriate. The selected goniolens was 2/3rds filled with a coupling gel, typically either carbomer gel 2mg/g (Viscotears; Novartis Pharmaceuticals) or hypromellose 1%, (Alcon; Belgium) before placing in contact with the cornea, ensuring neither air bubble nor nictitating membrane entrapment. For FCR-UK dogs, the ICA was viewed using a handheld slit-lamp biomicroscope (Kowa SL-14 or SL-15). For FCR-Swiss dogs the ICA was viewed using either a handheld slit-lamp biomicroscope (Clement Clarke BA904) or a Genesis-D fundus camera. All examinations were performed on conscious dogs, without sedation or pharmacological mydriasis and ensuring bilateral gonioscopic examination of the entire 360° of the ICA.

The ICA was primarily examined for presence or absence of PLD, determined by abnormally broad and thickened pectinate ligament fibres or solid sheets of pectinate ligament tissue, with or without 'flow-holes' but lacking in normal interfibre spaces. Where PLD was present, this was quantified by assigning a percentage of the 360° affected, determined after systematically viewing the entire circumference of the ICA.

Comparison of results between first and second gonioscopic examinations aimed to identify any progression of PLD, effectively testing a null hypothesis of 'no change in degree of PLD' for each dog. It was important to quantify the severity of any progression without overly interpreting a small ~~change which~~change, which could be influenced by subjectivity. This was achieved by first simplifying the PLD percentage to a grade, according to an ordinal scale ranking as follows:

- 0 - Not affected
- 1- Affected, mild PLD involving <20% of ICA
- 2- Affected, moderate PLD involving 20% - 90% of ICA
- 3- Affected, severe PLD involving more than 90% of ICA

Any change in the grade of PLD between first and second gonioscopic examinations, was then determined. Mild progression of PLD was determined as a one-step increase in the ordinal scale, i.e. from grade 0 (unaffected) to 1, from 1 to 2 or from 2 to 3. Moderate progression of PLD was defined as either progression from grade 0 to 2 or 1 to 3. Severe progression of PLD was defined as an increase from grade 0 to 3.

The results were statistically analysed using a one-tailed, paired t-test using GraphPad Prism[13] to establish if change was significant, with a p value of <0.005. The time period between first and second gonioscopic examinations for each dog was also evaluated.

RESULTS

Data for first and second gonioscopic examinations was recorded for 39 dogs in the FCR-UK group, (17 males, 22 females) and 57 in the FCR-Swiss (27 males, 30 females), the details of which are noted in Table 1.

In the FCR-UK group, the time interval between first and second gonioscopic examinations ranged from 1.92 to 12.58 years, (mean of 6.65 years, median 6.58 years). In the FCR-Swiss group, the time interval between first and second gonioscopic examinations ranged from 3.25 to 11.17 years, (mean of 5.68 years, median of 5.25 years). Date of the original certifying examination could not be verified for one FCR-Swiss dog, dog 30. Examination interval mean for both populations was 6 years, median 5.75 years.

Age of the dogs at second examination ranged from 4.58 to 12.75 years in the FCR-UK, with a mean of 8.4 years and median of 8.08 years. In the FCR-Swiss the age of examined dogs ranged from 2.25 to 13 years, with mean of 7.04 years and median of 6.83 years.

The results of the first and second examinations from each group of dogs are summarised in Table 2. This demonstrates that 14/96 dogs were noted as PLD-affected at the first examination. In total, 11/96 were classified as grade 1 and 3/96 as grade 2, comprised of 3/39 FCR-UK dogs (all grade 1) and 11/57 FCR-Swiss dogs (8/57 grade 1 dogs and 3/57 grade 2). At the second examination, 45/96 dogs exhibited PLD. Of these affected dogs, 17/39 were from the FCR-UK group (8/39 were grade 1, 5/39 grade 2, 4/39 grade 3) and 28/57 were FCR-Swiss (4/57 grade 1, 12/57 grade 2, 12/57 grade 3). In total, 12/96 dogs were grade 1 PLD-affected, 17/96 were grade 2 and 16/96 were grade 3.

Comparison between first and second examinations revealed progression of PLD grade in 39/ 96 (40.6%) of the total dogs (Table 3) and this change was determined to be highly statistically significant ($p<0.0001$). Of those dogs demonstrating progression, 13/96 (13.5%) exhibited mild progression, comprised of 6/39 of the FCR-UK dogs and 7/57 of the FCR-Swiss. Moderate progression was observed in 14/96 (14.6%) dogs, including 6/39 FCR-UK and 8/57 FCR-Swiss. Severe progression was exhibited by 12/96 (12.5%) dogs, of which 3/39 were FCR-UK and 9/57 FCR-Swiss. As it could be argued that those which demonstrated only a mild degree of progression could have been influenced by examination subjectivity the statistical analysis was repeated after eliminating 'mild' progression (changing these to 'no progression'), effectively including 'moderate' and 'severe' as the only relevant PLD progression. This revealed progression in 26/96, or 27.1% of dogs and this change in PLD grade was still found to be highly statistically significant ($p<0.0001$). Comparing the two populations, 9/39 (23.1%) of FCR-UK dogs demonstrated this moderate or severe progression and 17/57 (29.8%) of FCR-Swiss.

Fifty-seven dogs (59.4%) demonstrated no progression in PLD between first and second examinations. Of these, 24 dogs were from the FCR-UK group and 33 from the FCR-Swiss group. Two of the 24 FCR-UK dogs were grade 1, the remainder unaffected (grade 0); 2/33 of the unchanged FCR-Swiss dogs were grade 1 and 2/33 were grade 2, with the remainder unaffected.

Additionally, by the second examination two of the dogs had developed clinical glaucoma, one from each of the FCR-UK and FCR-Swiss groups. Both exhibited a PLD grade of 3, i.e. 90% or more of the ICA affected by PLD, at the second examination. The FCR-Swiss dog (number 57) had undergone a moderate PLD progression, from grade 1 to grade 3. The UK dog (dog number 39) had been described as 'unaffected OU' at first examination and severe progression was noted OS at second examination. The contralateral eye could not be evaluated as it had previously been enucleated due to a clinical diagnosis of intractable primary glaucoma. Histopathology was not available for inclusion but it was this report of an individual case of primary glaucoma in a certified 'unaffected' dog that had prompted this study.

DISCUSSION

To the authors' knowledge, this is the first report describing evolution of pectinate ligament dysplasia in individual dogs over time, in a breed affected by primary, angle closure glaucoma.

As already described, clinical reports of glaucoma in FCR dogs previously certified as 'unaffected' or <20% affected had incentivised the study. It was also ideal to look at a breed with an established link between PLD and glaucoma and in which glaucoma has been demonstrated to be hereditary. In the 1998 paper by Read *et al*, examining a random population of 398 Flatcoated Retrievers in the UK, incidence of PLD was determined as 34.7%, compared to 6% in a control population of 100 dogs of various other breeds[3]. Data from the BVA/KC/ISDS Eye Scheme over the period 2007-2011 shows an incidence of PLD of 5.38%, suggesting a significant reduction in PLD incidence since

initiation of gonioscopy screening for this breed (BVA, personal communication, December 2012).

Read *et al* also examined an additional target population of 48 FCR (either relatives of high scoring PLD individuals or those presenting with PLD associated with glaucoma) and the relationship between age as a covariate and incidence of glaucoma was investigated. Age was found to be insufficient as an individual variable to account for glaucoma, in the face of the variable PLD, although mean PLD grade did increase with FCR age[3]. In another survey, of 279 normotensive English Springer Spaniel dogs in Norway, a PLD prevalence of 25.5~~% was~~% was determined as well as a narrowing of relative width of ciliary cleft (RWOCC) in 17.9%[14]. Five of those dogs went on to develop glaucoma and an additional 9 dogs with glaucoma were also examined and a clear relationship was established between glaucoma and both narrowing of the RWOCC and degree of PLD. In this population a positive relationship between both age and PLD grade was established on narrowing of the RWOCC. Both of these studies contrast to ours in that they were cross-sectional rather than longitudinal however the positive correlation noted between age and severity of PLD supports our findings, that PLD in some individuals advances with age.

The gonioscopy examination and interpretation method used here was based on that used by Read, which determined the ICA percentage affected by PLD before simplifying that percentage to an ordinal scale ranking[3]. The scale used by Read differed from ours in defining unaffected as <25% PLD, with a nominal value of 12.5%, so as not to overemphasise any potential normal ICA variation. In our study it was preferable to use a value of <20% as grade 1 as this correlates with a level of ICA variation considered acceptable for hereditary eye screen examinations in the UK[11]. Quantifying the higher percentages of PLD in to a meaningful ordinal scale ranking was important but it was also necessary to not overly interpret percentages of PLD, considering the degree of subjectivity inherent to gonioscopy. This was particularly important considering comparisons were being made between a prospective or contemporary second examination and an historical first examination. The broad categorisation of grade 2 as between 20% and 90% represented clinically relevant PLD. Grade 3, at >90% was consistent with a near total ICA abnormality and a high risk of glaucoma[3].

Consistency of examination technique between first and second examinations was also imperative. All examiners were experienced eye panel ophthalmologists (BVA/KC/ISDS for the UK, ECVO for Switzerland). In the Swiss population consistency was aided by virtue of maintaining the same examiner. In the FCR-UK this was not stipulated prior to the study, due to concern that this may limit data available. However, due to the stringent membership requirements of the BVA/KC/ISDS eye panel (all panellists are experienced ophthalmologists who undergo a rigorous examination procedure prior to their acceptance on the panel), variation in technique and expertise with regard to gonioscopy and quantifying PLD should be minimal. During subsequent analysis of the first examination data it also transpired that the three eye panellist examiners who performed the second examination had also performed two-thirds (26/39) of those first

examinations. At the second examination it was also ensured that at least two, if not all three of the examiners, looked at each FCR-UK dog with any PLD abnormality, thus ensuring consistency of examination technique and agreement of results.

Evolution of pectinate ligament dysplasia was noted in 39/96 (40.6%) of the total population of Flatcoated Retrievers and statistical evaluation of this change determined it to be highly significant. Of these, 15/39 (38.5%) were FCR-UK and 24/57 (42.1%) were FCR-Swiss. So as to eliminate over-interpretation of results the statistical analysis was repeated, with an assumption that those ~~dogs which demonstrated only 'mild' progression~~dogs, which demonstrated only 'mild' progression, may not have demonstrated clinically relevant change or could have been influenced by examiner subjectivity. It was still shown that 26/96 (27.1%) dogs demonstrated progression of ~~PLD which~~PLD, which was still highly statistically significant.

Further investigations are required both to further quantify these ICA changes and investigate the mechanism involved in this change in the pectinate ligament. Utilising high-resolution ultrasound biomicroscopy and/ or ultrasound biomicroscopy would allow assessment of the deeper recess of the ciliary cleft for each individual with digital recording for comparison of results, potentially reducing subjectivity and improving inter-examination reproducibility[15]. However, these imaging modalities provide a cross-sectional view of the ICA, effectively perpendicular to the radial pectinate ligament itself and it may be that this does not necessarily allow good quantification of circumferential PLD specifically.

As pectinate ligament structure is fully developed by 8 weeks postnatally[10] the progression of pectinate ligament 'dysplasia' over time must occur due to other factors. Possible mechanism of this change may be a flattening of the pectinate ligament over time, a unification between primary (anterior) and secondary ligaments or other altered structure due to degenerative process. Although Samuelson and Gelatt[10] considered the ICA morphology to be mature at 8 weeks of age, increased numbers of trabecular cells including melanocytes were noted later at 12 and 16 weeks and penetration of the pectinate ligament collagenous core deep into Descemet's membrane became progressively harder to identify due to increased envelopment by that thickening membrane. If progressive cellular deposition around the collagenous core of pectinate ligaments is continued throughout life the ligament becomes increasingly thickened, forming wider sheets of tissue. Where there is an association with narrowing of the ICA, this could physical compress or spread the pectinate ligament or contribute to reduced surface area available for cellular deposition. A relationship between PLD and narrowing of the ICA was indicated histologically in the Bouvier des Flandres breed by van der Linde-Sipman in 1987[16]. Deposition of periodic acid-Schiff (PAS) positive material, akin to thickened basal membrane, was also noted on the trabecular meshwork and behind the primary pectinate ligament, in both glaucomatous and severely-PLD affected eyes. Extensive basal lamina–like material including heparan sulfate type proteoglycans has been noted to accumulate in the trabecular meshwork in human forms of glaucoma including goniodysgenetic glaucoma[17].

Ekesten *et al* scored the width of the ICA in his gonioscopic study of PLD in the Samoyed as well as the English Springer Spaniel and in both noted a progressive narrowing of the ICA with age[14, 18]. The examiners in this study did not score angle width as it was considered too subjective to be evaluated particularly as it had not been quantified in the first examination. Read *et al* had not evaluated width of ICA in the 1998 study, considering it too highly variable, including between eyes in individual dogs and even within the same eye[3]. However, of the FCR-UK group, it was noted at second examination that four dogs had 'narrow' ICA (dogs 7, 12, 18, 22) and one had 'closed' ICA (dog 37). None of these had either PLD or abnormal angles described on first examination data. Severity of PLD grade at second examination did not necessarily appear to correlate to the described width, although this could not be statistically evaluated due to the small numbers described affected. One was classified as PLD grade 1, two as grade 2 and one as grade 3 for the 'narrow' angles; the 'closed angle' dog was described as grade 2; these were all normotensive.

It is important to consider that gonioscopy also allows examination of peripheral anterior synechiae and these may readily be misinterpreted as PLD by less experienced examiners. These are typically broad-based at the iris and not within the ICA itself but anterior to and obscuring it The dogs in the current study had no history of antecedent ocular disease and were examined by experienced ophthalmologists, thus this is unlikely to be a confounding factor. At the time of examination, all but two were normotensive, thus in only those two was it possible that clinical disease had modified the ICA. A difference was not described in PLD morphology of the ~~two which~~two, which did exhibit glaucoma.

Pectinate ligament appearance can also change with spread of pigment from the anterior surface of the iris, as seen with ocular melanosis in the Cairn Terrier, which is most pronounced ventrally[19]. Bjerkas and Ekesten speculate that subclinical inflammatory events could have been responsible for the progression of PLD with age in their population of ESS dogs. In the Basset Hound uveitis frequently accompanies glaucoma and is suspected to contribute to deterioration of ICA conformation with time[1]. Bedford ~~described abnormal~~described abnormal pectinate ligament structure in the glaucomatous Basset, with a narrowed and darkly pigmented ICA[6, 7]. Histopathologically, the closed ciliary cleft was covered by an extension and reflection of Descemet's membrane and he surmised that part of the amorphous material noted gonioscopically was aggregations of Descemet's membrane. Gonioscopy had revealed sheets of grey-white amorphous tissue with broad iris-bases crossing the ICA and these may also have been more akin to peripheral anterior synechiae.

The clinical relevance of this study ultimately arises as we consider the (12/96) dogs which demonstrated severe progression of PLD grade, from unaffected to grade 3. PLD of >90% is highly associated with a risk of glaucoma[3] and in fact two of the dogs in the current study had already developed glaucoma. This indicates that performing gonioscopy once only and at a young age as part of hereditary eye screen examinations underestimates the number of dogs which will go on to be at high risk of glaucoma. Wood *et al* demonstrated a hereditary basis for PLD and a hereditary basis for risk of

primary glaucoma in the breed, in a population of FCR of varied ages[4]. Our study suggests when performing hereditary eye screening and certifying PLD grade of <20% this underestimating those numbers of dogs who will have a hereditary risk of PLD in their progeny. The relevance of ICA comparisons between different dogs ~~need~~needs therefore to take account of age. We also now aim to perform inheritance ~~and genetic~~and genetic studies for these affected dogs, ideally with an expanded population of FCR.

The dogs in this study were effectively a random selection of normal FCR dogs, aiming to represent populations in both the UK and Switzerland; only dogs determined unaffected or grade 1 affected at first examination however re-presented. Inevitably, a selection pressure occurs due to the willingness of breeders and owners to participate. Further studies examining pectinate ligament change in a cohort of dogs with more severe PLD over a period of time would be useful, as the incidence of progression of PLD grade may differ.

In summary, this study indicates evolution from normal ICA architecture to clinically significant PLD in individual dogs, over a period of time. Gonioscopic examination has become an essential component of hereditary eye screening within the UK BVA/KC/ISDS and ECVO hereditary screening and potentially this study suggests that this should not be a 'once in a lifetime' examination.

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REFERENCES

1. Gelatt KN, Brooks DE, Källberg ME. The Canine Glaucomas. In: *Veterinary Ophthalmology* 4th edition (ed. Gelatt KN). Blackwell Publishing. Iowa, 2007; 753 -811
2. Gum GG, Gelatt KN, Esson D. Physiology of the Eye. In: *Veterinary Ophthalmology* 4th edition (ed. Gelatt KN). Blackwell Publishing. Iowa, 2007; 149- 182
3. Read RA, Wood JLN, Lakhani KH. Pectinate Ligament Dysplasia (PLD) and glaucoma in Flat Coated Retrievers. Part 1: Objectives, technique and results of a PLD survey. *Veterinary Ophthalmology* 1998; **1**: 85-90
4. Wood JLN, Lakhani KH, Read RA. Pectinate Ligament Dysplasia (PLD) and glaucoma in Flat Coated Retrievers. Part 2: Assessment of prevalence and heritability *Veterinary Ophthalmology* 1998; **1**: 91-99

5. Martin CL. Gonioscopy and Anatomical Correlations of the Drainage Angle of the Dog *Journal of Small Animal Practice* 1969; **10**: 171-184
6. Bedford PGC. The aetiology of primary glaucoma in the dog. *Journal of Small Animal Practice* 1975; **16**: 217-239
7. Bedford PGC. A gonioscopic study of the iridocorneal angle in the English and American breeds of Cocker Spaniel and the Basset Hound. *Journal of Small Animal Practice* 1977; **18**: 631-642
8. Bedford PGC. Gonioscopy in the dog. *Journal of Small Animal Practice* 1977; **18**: 615-629
9. Martin CL. The normal canine iridocorneal angle as viewed with the scanning electron microscope. *Journal of American Animal Hospital Association* 1975; **11**:180-192
10. Samuelson DA, Gelatt KN. Aqueous outflow in the Beagle. I. Postnatal morphologic development of the iridocorneal angle: pectinate ligament and uveal trabecular meshwork. *Current Eye Research* 1984; **3**:783-794
11. British Veterinary Association/ Kennel Club/ International Sheep Dog Society Eye Scheme. Information for Panellists. Part III Technical Information. Inherited Ocular Disease: Breeds Affected and type of condition
12. ECVO Presumed Inherited Eye Diseases. Revised 2010-05-27 Berlin. www.ecvo.org
13. GraphPad Prism Version 5.01, August 7, 2007 for Windows; GraphPad Software, Inc., California, USA
14. Bjerkas E, Ekesten B, Farstad W. Pectinate ligament dysplasia and narrowing of the iridocorneal angle associated with glaucoma in the English Springer Spaniel. *Veterinary Ophthalmology* 2002; **5**: 49-54
15. Bentley E, Miller PE, Diehle KA. Evaluation of intra- and interobserver reliability and image reproducibility to assess usefulness of high-resolution ultrasonography for measurement of anterior segment structures of canine eyes. *American Journal of Veterinary Research* 2005; **66**:1775-1779
16. Van der Linde-Sipman JS. Dysplasia of the pectinate ligament and primary glaucoma in the Bouvier des Flandres dog. *Veterinary Pathology* 1987; **24**:201-206.
17. Tawara A, Inomata H. Distribution and characterisation of sulphated proteoglycans in the trabecular tissue of goniodysgenetic glaucoma *American Journal of Ophthalmology* 1994; **117**:741-755
18. Ekesten B, Narfström K. Correlation of morphological features of the iridocorneal angle to intraocular pressure in Samoyeds. *American Journal of Veterinary Research* 1991; **52**: 1875-1878
19. Petersen-Jones SM, Forcier J, Mentzer L. Ocular melanosis in the Cairn Terrier: clinical description and investigation of mode of inheritance. *Veterinary Ophthalmology* 2007; **10**:63-69